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Effects of Experience on Maternal Behavior in
Belding's Ground Squirrels (*Spermophilus beldingi*):
Older and Wiser?

A Thesis Submitted

by
Mikael D. Langner

In Partial Fulfillment of the Requirements
for the Degree
Master of
Biology

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ABSTRACT

Effects of Experience on Maternal Behavior in Belding's Ground Squirrels (*Spermophilus beldingi*): Older and Wiser?

By

Mikael D. Langner

Maternal behavior is an important behavior because it influences whether or not raising one's offspring is successful. Maternal experience is positively correlated with successful maternal behavior. This association is evident when comparing first time mothers with mothers who have prior experience raising young. Life experience not related to raising young can also positively influence maternal behavior. In this work, the hypothesis that is tested is whether or not maternal experience enhances the ability of a female to assess the threat level of a situation. The model species used to conduct the study is the Belding ground squirrel (*Spermophilus beldingi*), and the study is conducted in an alpine meadow near Yosemite's Tioga Lake, which is the native habitat of the model species. To test the hypothesis, the research team conducts intruder tests in which an unfamiliar male in a cage is placed in a female's maternal territory. Responses to the intruder male are recorded for multiparous adult, primiparous adults, and primiparous yearling females. As the breeding season progresses, all three female types learn to respond appropriately, but in the early breeding season, the primiparous yearling females do not show appropriate cautionary activity toward the intruder. Instead, they are passive, and they only observe the intruder male. However, primiparous adults do also show appropriate activity early in the breeding season, which indicates that maternal experience can also be learned through life experience.

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INTRODUCTION

Maternal experience in mammals has been shown to have an array of effects on behavior that might increase reproductive success. The effects of experience are evident when comparing behavior between mothers who are raising young for the first time (primiparous) and mothers who have prior experience raising young (multiparous).

For example, prior experience as a mother alters vocalizations and enhances bonding with infants in sheep (*Ovis aries*; Dwyer et al., 1998; Dwyer and Lawrence, 2000; Viérin and Bouissou, 2002). Multiparous sheep have low-pitched vocalizations in comparison with primiparous sheep, and this low-pitch reflects not only physiologic changes in pregnancy, such as increased oxytocin levels, but also a more mature neurosensory system in multiparous sheep compared to primiparous sheep. As both classes of sheep have increased oxytocin levels at birth, they both initially have low-pitched vocalizations, which are heard in sheep only at the time of parturition and the immediate after period because young sheep are known to bond with their mothers in response to these low-pitch vocalizations whereas primiparous sheep have high-pitched vocalizations with straying of their young. The end result is that young sheep stray more with high-pitched vocalizations than with low-pitched vocalizations because the young sheep bond more with mothers who emit low-pitched vocalizations. Primiparous mothers may have immature neurosensory systems that do not permit them to make this association between bonding and low-pitched vocalizations; they sense greater danger from prey with straying of their young but emit only high-frequency vocalizations in this situation, rather than the more highly developed multiparous response which recognizes that bonding—and thus keeping their young nearby—depends on low-pitched vocalizations. Likewise, the fear response in *O. aries*

mothers decreases as they gain maternal experience (Vierin and Bouissou, 2002), and their low-pitched vocalizations may reflect this decreased fear. Multiparous sheep have less fear of humans than nulliparous or primiparous sheep; in this study, humans were immobile and offered a food reward, which may account for the loss of fear in the multiparous sheep, who were able to recognize this situation as non-threatening. In addition, sheep were assessed for their response to a human startle stimulus, and again the same results were found, with multiparous sheep being best able to assess the situation as non-threatening and primiparous and nulliparous maintaining the greatest distance from the stimulus. Thus, maternal experience enhances coping ability in *O. aries* (Meurisse et al., 2005), while primiparous female *O. aries* show more maternal rejection behaviors than do multiparous females, perhaps due to their younger age, greater anxiety, and neophobia at the presence of the lamb.

In rhesus monkeys (*Macaca mullata*), maternal experience reduces submissive interactions with conspecifics (Hooley and Simpson, 1981). Moreover, primiparous female *M. mullata* are more excitable and less confident than older, multiparous mothers. The increased excitability might have resulted in part from the lack of familiarity primiparous females had with human observers during the study. Thus, lack of maternal experience combined with novelty in the environment appears to lead to greater excitability in primiparous mothers.

In prairie voles (*Microtus ochrogaster*), prior experience as a mother enhances care of young (Wang and Novak, 1994). Enhancement of care may be related to learning: mothers with prior litters have had more opportunities to learn mothering skills, and thus offer enhanced maternal care to their young, whereas inexperienced mothers have not had as many chances to hone their maternal skills and behavior. Male *M. ochrogaster* show the same parenting skills

regardless of their experience caring for young. Thus, enhancement of parenting skills seen in female *M. ochrogaster* may be related to sex specific factors.

Reproductive success in Weddel seals (*Leptonychotes weddellii*) is influenced by the ability of mothers to obtain a high-quality nesting site (Hastings and Testa, 1998). For example, pups born in outer Erebus Bay in McMurdo Sound, Antarctica, have greater mortality than do pups born in inner Erebus Bay, suggesting that site locations vary in harshness of environmental exposure. Mothers with prior maternal experience are more likely than inexperienced mothers to establish nesting sites in inner Erebus Bay. Nesting sites are plentiful in both the inner and outer bay, suggesting that inexperienced mothers understand the difference in quality between the two locales and select the higher quality sites.

Cameron et al. (2000) observed in female horses (*Equus caballus*) that older mares were more protective of offspring than younger mares during the first twenty days after birth when offspring are the most vulnerable, but less diligent thereafter, and were also more likely to give birth in consecutive years. They suggested that experienced mothers are better able than inexperienced mothers to optimally channel reproductive investment, and thus are better able to maximize reproductive success.

Among human mothers with post-partum depression, multiparous women touch and hold their infants to a greater extent than do first time mothers (Ferber, 2004). It is thought that less confidence portends less touching and holding behavior for inexperienced human mothers. The highest rates of holding were observed among multiparous women without post-partum depression. Mothers with maternal depression showed the lowest rates of holding regardless of parity status, perhaps due to a withdrawal state induced by the depression. In the middle were multiparous women with maternal depression, who showed many of the same behaviors as their

multiparous counterparts without depression, which may be due to their observations of other mothers with their infants.

Breeding experience also appears to increase the proficiency of behavior in males of some mammalian species. For example, the amount of time male black-tufted-ear marmosets (*Callithrix kuhlii*) devote to carrying their infants increases as they gain experience caring for young, and this change is associated with decreased testosterone levels in males (Nunes et al., 2000a, 2001). Moreover, although male California ground squirrels (*Spermophilus beecheyi*) do not contribute to caring for young, the proficiency of their mating behaviors improves with experience (Holekamp and Talamantes, 1992).

Effects of experience on maternal behavior are also apparent when comparing females who have raised young in the past or are currently raising young (parous) with females who have never been a mother (nulliparous). For example, parous female rats (*Rattus norvegicus*) show increased mobility in their home territories than do nulliparous rats, which appears to result from improved spatial memory and greater exploratory behavior in parous females (Love, et al., 2005).

In nulliparous female *R. norvegicus*, exposure to pups induces maternal behavior, and females maintain a maternal memory that shapes subsequent behavior (Scanlan et al., 2006). That is, they quickly display maternal behavior when later exposed to foster pups again. However, maternal memory and neurochemical responses associated with maternal behavior are stronger in female rats who have given birth, suggesting that pregnancy and parturition enhance the development of maternal memory. Thus, both pregnancy and exposure to pups appear to be important in the development of behavioral changes observed between primiparous and nulliparous females.

A variety of studies have identified changes in the brain associated with obtaining maternal experience in mammals. These neural changes may be triggered by pregnancy, parturition, or the experience of caring for young, and many have been suggested to increase the proficiency of maternal behavior. For example, estrogen receptor densities in the hypothalamus and limbic regions of the brain during late gestation are higher in multiparous than primiparous *O. aries*, and increased receptor density may help facilitate the expression of maternal behavior after lambs are born (Meurisse et al., 2005). Multiparous female *R. norvegicus* are more responsive to prolactin than primiparous females due to upregulation of prolactin receptors in preoptic area of the hypothalamus, which is associated with maternal care behaviors (Anderson et al., 2006). Release of neurotransmitters in the olfactory bulb as a result of vaginocervical stimulation during parturition is greater in multiparous than primiparous *O. aries*, and this greater release of neurotransmitters has been suggested to enhance olfactory recognition of newborn lambs and strengthen maternal bonding (Lévy et al., 1995). In *R. norvegicus*, gestation and parturition increase the responsiveness of females to foster pups (Scanlan et al., 2006), and experiences associated with motherhood decrease neural activation in areas of the hippocampus and amygdala associated with responsiveness to environmental stressors, increasing boldness and decreasing fearful behavior while females are raising young (Wartella et al., 2003). Moreover, in *R. norvegicus*, maternal experience causes modifications in the hippocampus that generate long-term improvement in learning and memory (Kinsey et al., 1999; Pawluski et al., 2006).

A study of human mothers examined the maternal behavior of three age groups: teens, young mothers, and mature mothers from 26 to 40 years old (Krpan et al., 2005). Teen mothers have been previously shown to control their infants in unhealthy ways. It is also known that 30%

of mothers who were abused as children abuse their own children, but only 5% of mothers from non-abusive upbringings become abusers of their infants. Children who experience maternal rejection grow up to reject their own offspring. It is also thought that expression of maternal behavior is under the control of hormones, neuropeptides, and their receptors.

Krpan et al. (2005) noted that the hypothalamic-pituitary-adrenal axis is one system affected by early experiences. The hypothalamus and pituitary regulate at least six major hormone systems, with one example being ACTH stimulation of cortisol release. Cortisol is usually known as the stress hormone, but in healthy mothers it is related to arousal, attention, and increased awareness. Likewise, abnormally low levels of ACTH in pregnant teens often causes depression in early and late pregnancy and can lead to conduct disorder in early pregnancy and post-partum. In mature mothers, however, high cortisol levels are associated with negative moods. In the end, this study explored the differences between teen and adult maternal behavior, and the role of maternal age cortisol and early life experiences in the regulation of maternal behavior and post-partum mood state.

Demographic characteristics of the population studied indicate that the teens were less financially stable and unmarried in comparison to their older counterparts. One possible confounding factor in this study is the social support provided to the pregnant teens. This support may have offset the partners' and relatives' support of older mothers, whereas many pregnant teens lack such support. The median income of teens was \$8000 with 83% involved in social services. The median income of older mothers was \$30,000 with 0% involved in social services.

Krpan et al., (2005) also noted that caregiver transitions and parental separations have been shown to predict adult psychopathology. Caregiver transitions can include moving back

and forth between parents homes, living in foster care, and living with other family members (Krpan et al., 2005).

To observe maternal behavior with infants, Krpan et al. (2005) videotaped mothers for 15 minutes. This interaction was a single, private, non-feeding interaction. Krpan et al. (2005) concede that this period of time is insufficient to permit generalizability to maternal behavior throughout the offspring's life. Teen mothers spent more time cleaning the infant, fixing clothes and changing diapers, while older mothers were more affectionate, often kissing their children. This study pointed out that mature mothers also engaged in instrumental behavior like the teens, and that teens also engaged in affectionate behavior.

Life experience not related to raising young can also influence maternal behavior. For example, life experience enhances coping ability, but to a lesser extent than maternal experience. Animal studies demonstrate dopamine release in response to aversive stressful stimuli (Pruessner et al., 2004). While the study cited the animal studies as the basis for their own study, it actually screened 120 healthy young college students for parental care in early life using telephone surveys. Five students from the top end of care and five from the bottom end of parental care then had PET scans following a psychosocial stress task. The low parental care students had higher release of dopamine from the ventral striatum as measured by reduction in [11C] raclopride binding, suggesting less coping ability than high parental care students.

Optimal maternal behavior may result from a combination of maternal and life experience. Maternal behavior does not occur in a vacuum. Becoming an "efficient" mother involves many skills and behaviors, including foraging and returning to nest, which requires excellent spatial memory (Wartella et al., 2003). Both age and early life experience affect maternal behavior. Older mothers and mothers who received consistent care as children showed

more affectionate behavior to their infants than mothers who were younger or who had received inconsistent care as children. Young mothers or those who had received inconsistent care as children showed more instrumental behavior such as burping or wiping the baby and less affectionate behavior (Krupan et al., 2005).

Much prior work has focused on the ways in which behavior changes as females gain experience raising young. Some of these studies have also suggested that maternal experience influences the way females assess and cope with situations (e.g., Cameron et al., 2000; Wartella et al., 2003; Meurisse et al., 2005). In this work, I evaluated the hypothesis that maternal experience enhances the ability of a female to assess the threat level of a situation and respond appropriately, using Belding's ground squirrels (*Spermophilus beldingi*) as a model species.

To test this hypothesis, I conducted intruder tests in which an unfamiliar male was placed in a cage in a female's maternal territory, and compared responses to the intruder among multiparous adult, primiparous adult, and primiparous yearling females. Female *S. beldingi* maintain maternal territories while rearing young, and aggressively evict intruding squirrels or small predators such as weasels who wander onto the territories. During the breeding season, squirrels attempt to commit infanticide against the young of other squirrels to reduce future competition for their own young. Thus, defending a maternal territory is vital for a female squirrel to ensure survival of her offspring. (Sherman, 1977).

I predicted that if experience hones the capacity to respond aptly to situations, then experienced females should be able to determine that a caged male is not a threat and not respond to him, whereas inexperienced females should not be as savvy and should respond aggressively and vigilantly toward the intruder even though he is caged.

METHODS

Spermophilus beldingi is a diurnal species inhabiting alpine and subalpine meadows in the western United States. Squirrels hibernate 8-9 months each year from late summer through spring. During hibernation, squirrels remain in underground burrows and subsist on fat reserves acquired just prior to entering hibernation. Adult males (≥ 2 years old) emerge from hibernation in reproductive condition, whereas yearling males (1-year-old) typically do not become reproductively competent and do not mate. By contrast, both adult and yearling females may mate; however, some yearling females delay breeding until they are adults. Adults begin emerging from hibernation before yearlings, and males before females. Males begin mating as soon as the first females to emerge enter estrus, and females typically become estrous within a week after emerging from hibernation. Females mate only one day per year and thus bear at most one litter per year. Gestation lasts 24-25 days. Young remain underground in natal burrows during lactation and first emerge from the natal burrow when they are about 25-28 days old. Females establish maternal territories during gestation and defend these territories during lactation and after the emergence of young from the natal burrow against predators and conspecifics who may attempt to commit infanticide. All surviving male *S. beldingi* emigrate from the natal area before breeding, either during their first summer as a juvenile or the following summer as a yearling. Females typically remain in their natal areas throughout their lives, and ordinarily less than 10% emigrate to a new home area (Morton et al., 1974; Morton and Gallup, 1975; Sherman, 1977; Jenkins and Eshelman, 1984; Holekamp, 1984; Holekamp et al., 1984, Nunes and Holekamp, 1996; Nunes et al., 1999).

From 4 June to 20 August 2005 and 6 June to 23 August 2006, a population of *S. beldingi* was studied in a 20-hectare meadow (37° 55' N, 119° 15' W) near Tioga Pass in Mono County, California, U.S.A. Only females who successfully weaned a litter during the summer were included in the study. Subject animals were permanently marked with numbered metal ear tags (National Band and Tag Co., Newport, Kentucky, U.S.A.) and repeatedly captured in live-traps (Tomahawk Live-Trap Company, Tomahawk, Wisconsin, U.S.A.) baited with peanut butter. The fur of squirrels was dyed with unique symbols to facilitate visual discrimination between individuals. From atop rocks and ladders observers watched the maternal burrows of lactating females daily to determine the dates on which their young first emerged from the natal burrow. Young of all litters were trapped all within 2 days of their first appearance above ground. Juveniles were ear tagged and weighed at their first capture. Body mass of adult and yearling females was measured with spring balance scales (Avinet, Dryden, New York, U.S.A) during gestation, lactation, and within two days after the first appearance of their young above ground. Body mass of females was measured within two days prior to behavioral testing (see below). Females were classified as multiparous adults, primiparous adults, or primiparous yearlings. A multiparous adult was defined as one who had successfully weaned at least one litter in a prior year. A primiparous adult was defined as one who had not given birth in a prior year. All yearling females were defined as primiparous since females do not begin reproducing until they are yearlings. Data were available from several prior years, so it was possible to determine whether a female had weaned a litter in an earlier year, or had given birth in an earlier year, as evidenced by enlarged nipples and a sharp, sudden decline in body mass.

Intruder tests were performed for each female during gestation, lactation, and within four days after the first appearance of young above ground. For the tests, a male not residing within

the focal female's home area was trapped. Female's typically remain within 80 meters of the maternal burrow during the breeding period (Holekamp, 1984), so a male was defined as having a non-overlapping home range with the focal female if he was not regularly observed or trapped within 80 meters of the focal female's home burrow. The intruder male was left in the trap and placed within two meters of the focal female's maternal burrow. Prior to placing the male near the female's burrow, an observer coaxed the female down her burrow by quietly walking toward her. After the female emerged from her burrow, her responses to the intruder male were videotaped for 10 minutes. Taping began when a female first responded to a male or showed awareness of the intruder by looking at him. Tests were conducted throughout the day, between 0800 hours and 1700 hours. Males were kept in traps for no more than 90 minutes, and each male was weighed before being released at his site of capture.

Video tapes were later viewed to evaluate the responses of females to a caged intruder. Responses observed during tests were divided into five categories, and the amount of time during the ten-minute test that a female exhibited each class of response was calculated. The amount of time that a female spent near (within two meters) of the intruder was also calculated. The categories of female responses are described below and are based on prior descriptions of behavior in *S. beldingi* (Holekamp, 1986; Nunes et al., 2000b).

Acts aggressively toward intruder: The female lunges at the trap, pushes or tips the trap, tries to bite or attack the intruder through the wires of the trap, growls, or takes a threat stance with her tail shaking, fur bristled and teeth chattering.

Investigates intruder: The female sniffs the trap or intruder or walks around the trap while watching the intruder.

Acts vigilantly toward intruder: The female is oriented toward the intruder while posting (on hind legs with body erect), half-posting (on hind legs with body partly crouched), or resting alert (on all fours with head or upper body raised).

Watches intruder: The female is oriented toward the intruder while resting (on all fours with head and upper body not elevated).

Acts indifferently toward intruder: The female is not oriented toward the intruder and is engaged in an activity such as resting, feeding, gathering nesting material, or excavating a burrow.

The behavior exhibited by caged males during intruder tests was also evaluated to determine whether body mass of males influenced the outcome of tests. For example, large males might tend to be more aggressive than small males during tests, which might affect how females act around them. In this analysis, male behavior was evaluated only when a female was near (within two meters of the male) and might be influenced by his behavior. Two meters was chosen because males uniformly did not respond to females who were more than two meters away. The behaviors of males were divided into five categories, and the amount of time that each male devoted to a category of behavior was calculated as a proportion of the time that the female was near the male. The five categories of male behavior are described below.

Acts aggressively toward female: The male lunges toward the female, tries to bite or attach the female through the wires of the trap, growls, or takes a threat stance with his tail shaking, fur bristled, and teeth chattering.

Investigates female: The male sniffs the female.

Acts vigilantly toward female: The male is oriented toward the female and posts, half-posts, or rests alert.

Acts submissively toward female: The male backs away from the female, crouches or cowers with his head lowered, or turns on his back with his ventrum exposed to the female.

Acts indifferently toward female: The male rests in the trap and exhibits none of the responses described above.

Data were evaluated to determine whether they were distributed normally (Lilliefors test, $P > 0.05$). Data expressed as proportions that were not distributed normally were transformed using the angular transformation (transformed value = $1/\sin(\sqrt{\text{original proportion}})$) to data that were normally distributed (Lilliefors test, $P > 0.05$). Correlations analyses using Pearson's r were performed between female-intruder mass differences and rates of male behaviors to assess whether there were any associations between the mass of a male and his behavior around a female. Responses of females were evaluated with analysis of variance (ANOVA), with parity class (multiparous adult, primiparous adult, primiparous yearling) and phase of the breeding period (gestation, lactation, post-emergence of young) being independent factors. Post hoc comparisons between parity classes were performed with Tukey's tests. Post hoc comparisons between phases of the breeding period were performed with paired t -tests. We considered differences between groups to be significant when $P \leq 0.05$. Significance levels of multiple pairwise comparisons with paired t -tests were adjusted with the Bonferroni correction. Means values are presented as mean \pm 1 SEM. Values presented in graphs reflect actual and not transformed data.

RESULTS

Behavior of Intruder Males

To determine whether female mass influenced the behavior of caged males during intruder tests, which might in turn influence female behavior, I evaluated the relationship between female-intruder mass differences and male behavior. Males were omitted from this analysis if the female spent less than 15 seconds in the proximity (within one meter) of the male during the test. There were no significant correlations between mass differences and the investigative ($r = -0.044$, $P = 0.620$, $n = 131$), aggressive ($r = 0.143$, $P = 0.102$, $n = 131$), vigilant ($r = 0.020$, $P = 0.823$, $n = 131$), submissive ($r = -0.090$, $P = 0.304$, $n = 131$), or indifferent ($r = 0.094$, $P = 0.284$, $n = 131$) of males.

Behavior of Females

The amount of time females spent near the caged intruder during the 10-minute behavioral tests varied significantly over the course of the breeding season (Fig. 1, ANOVA, $F_{2,141} = 3.923$, $P = 0.022$); however, post-hoc tests did not reveal significant differences between specific phases of the breeding period. I observed no significant difference in time spent near the intruder among parity classes (Fig. 1, ANOVA, $F_{2,141} = 1.882$, $P = 0.156$), and no significant interaction between phase of the breeding season and parity class in this analysis (Fig. 1, ANOVA, $F_{4,141} = 1.378$, $P = 0.245$).

The amount of time that females acted aggressively toward the caged intruder during behavioral tests did not vary significantly over the course of the breeding season (Fig. 2,

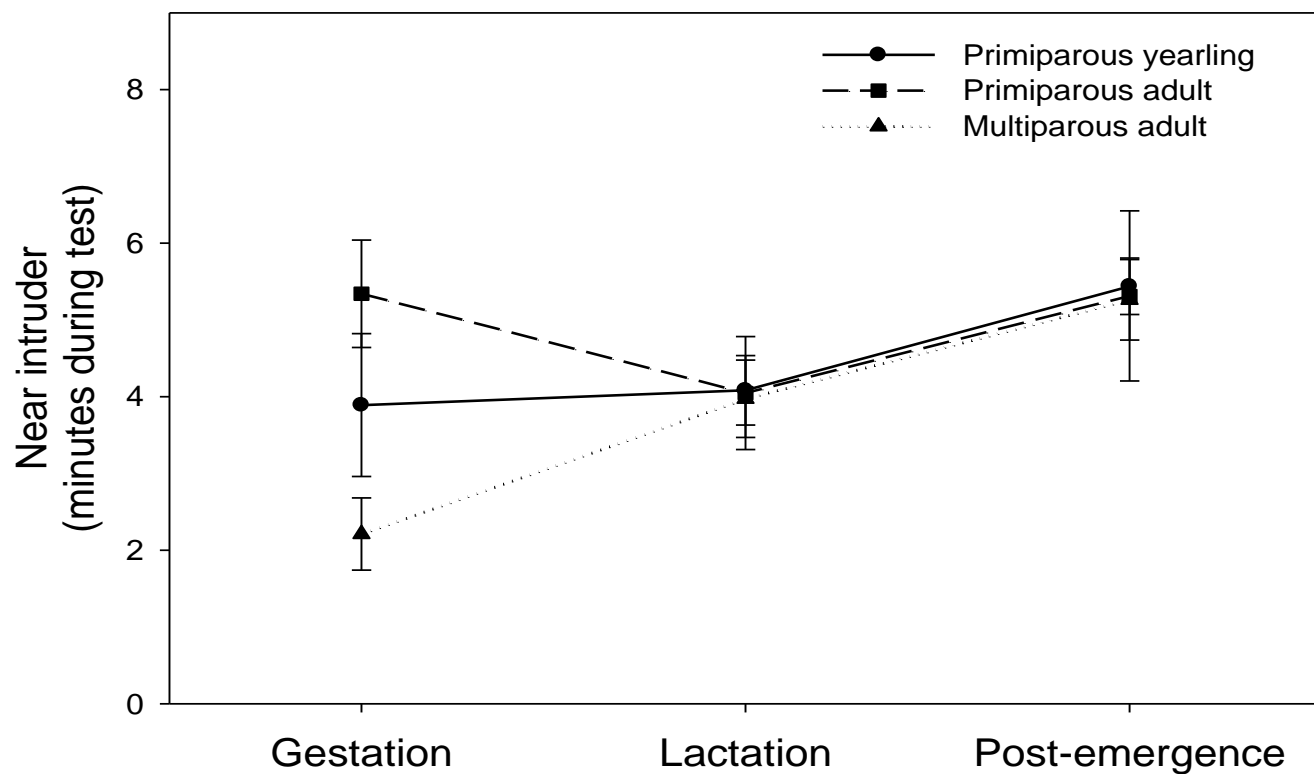


Figure 1. Time spent by female *S. beldingi* within one meter of a caged male intruder during a 10-minute behavioral test. Samples included 15 primiparous yearlings, 11 primiparous adults, and 13 multiparous adults during gestation, 15 primiparous yearlings, 16 primiparous adults, and 31 multiparous adults during lactation, and 12 primiparous yearlings, 16 primiparous adults, and 21 multiparous adults during the post-emergence period.

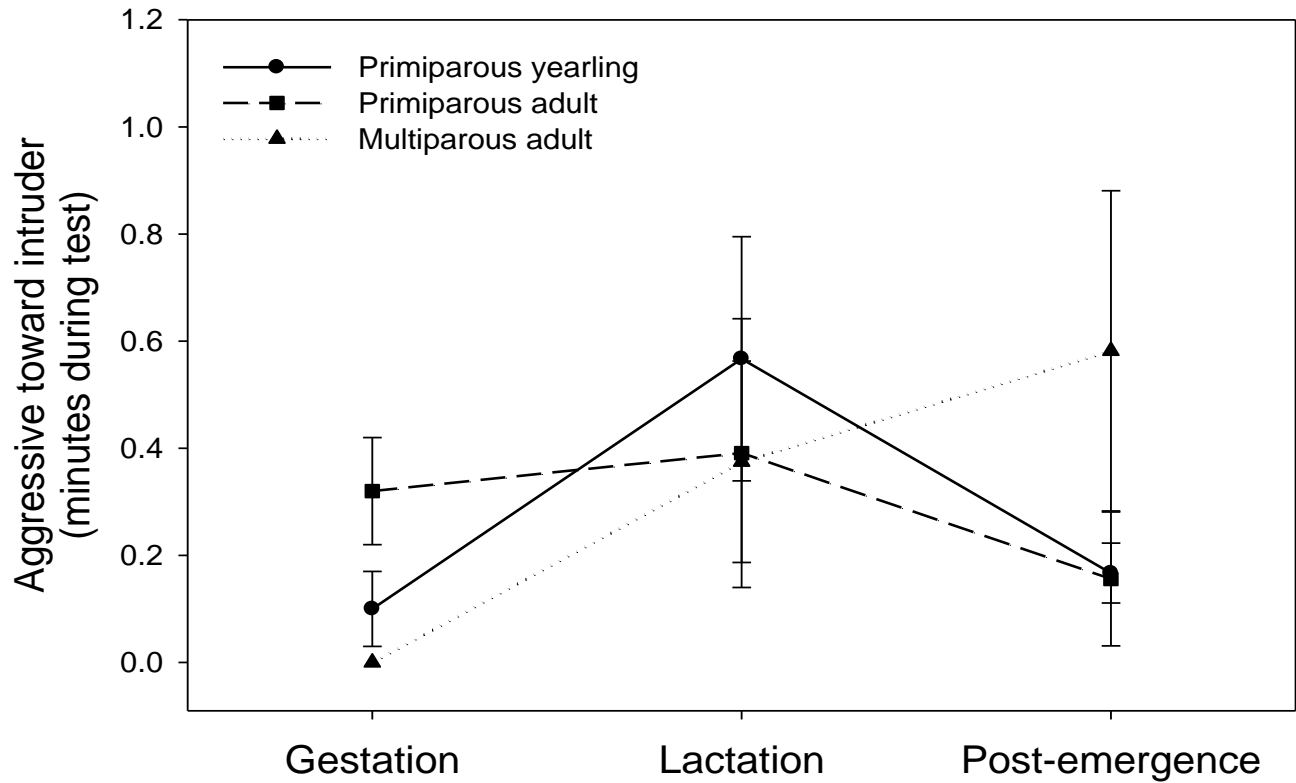


Figure 2. Time spent by female *S. beldingi* behaving aggressively toward a caged male intruder during a 10-minute behavioral test. Samples included 15 primiparous yearlings, 11 primiparous adults, and 13 multiparous adults during gestation, 15 primiparous yearlings, 16 primiparous adults, and 31 multiparous adults during lactation, and 12 primiparous yearlings, 16 primiparous adults, and 21 multiparous adults during the post-emergence period.

ANOVA, $F_{2,141} = 1.456$, $P = 0.237$) or among parity classes (Fig. 2, ANOVA, $F_{2,141} = 0.136$, $P = 0.873$), and I observed no significant interaction between phase of the breeding period and parity class in aggressive behavior (Fig. 2, ANOVA, $F_{4,141} = 1.449$, $P = 0.221$).

The amount of time females investigated caged males during intruder tests varied significantly with phase of the breeding season (Fig. 3, ANOVA, $F_{2,141} = 3.572$, $P = 0.032$) and with parity class (Fig. 3, ANOVA, $F_{2,141} = 3.855$, $P = 0.023$). I also observed a significant interaction between these two factors (Fig. 3, ANOVA, $F_{4,141} = 3.993$, $P = 0.004$), indicating that patterns of change in investigative behavior differed among groups of females over the course of the breeding period. In particular, during gestation investigative behavior was significantly greater in primiparous adults than in multiparous adults, with primiparous yearlings intermediate between these groups (Fig. 3). However, during lactation investigative behavior was significantly greater in primiparous yearlings than multiparous adults, with primiparous adults intermediate (Fig. 3). From gestation to lactation there was a significant decrease in investigative behavior among primiparous adults (Fig. 3). By contrast, investigative behavior increased significantly from gestation to lactation among primiparous yearlings, and then decreased significantly from lactation to the post-emergence period (Fig. 3).

The amount of time females behaved vigilantly during behavioral tests varied significantly over the course of the breeding season (Fig. 4, ANOVA, $F_{2,141} = 5.102$, $P = 0.007$), but not among parity groups (Fig. 4, ANOVA, $F_{2,141} = 0.873$, $P = 0.420$). I observed a significant interaction between phase of the breeding season and parity group (Fig. 4, ANOVA, $F_{4,141} = 3.973$, $P = 0.004$), indicating that patterns of change in vigilant behavior during the breeding period differed among parity groups. In particular, vigilant behavior was significantly

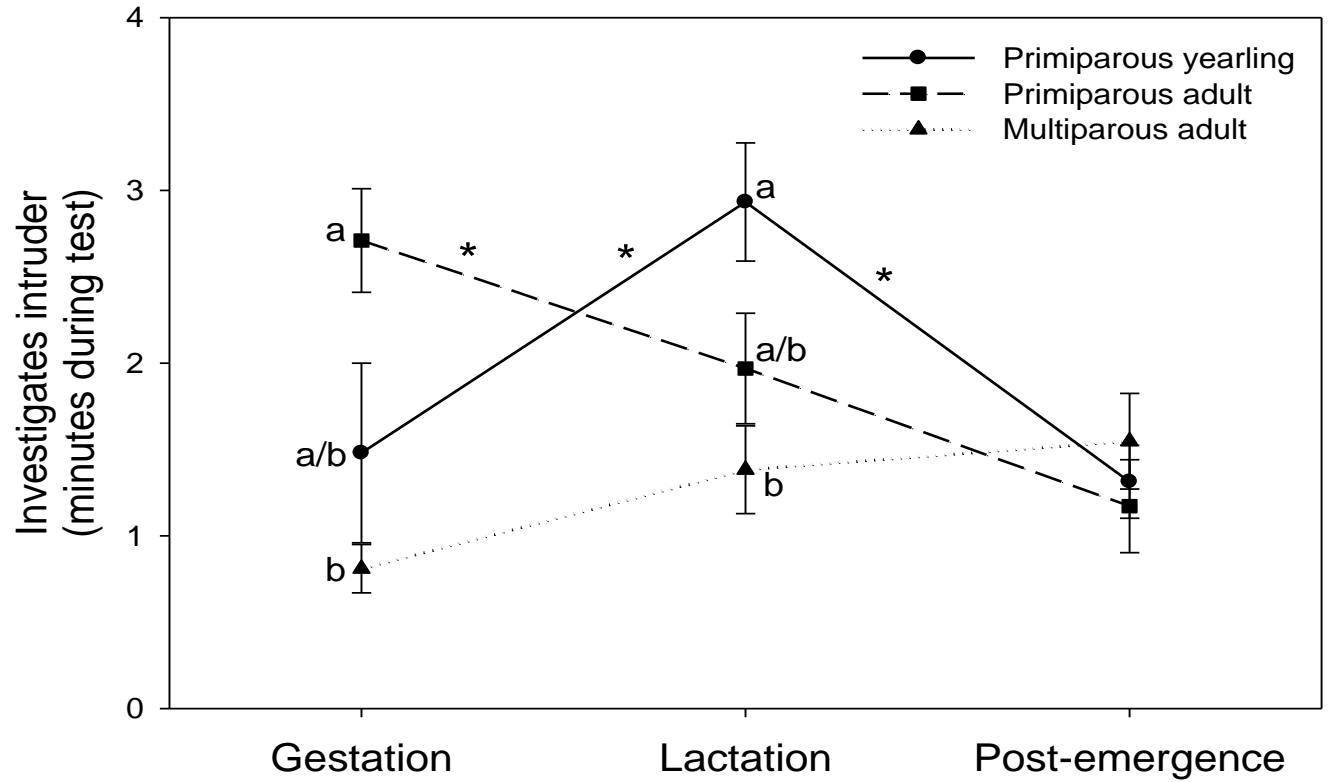


Figure 3. Time spent by female *S. beldingi* investigating a caged male intruder during a 10-minute behavioral test. Different lower-case letters next to symbols indicate a significant difference between parity groups within a phase of the reproductive period. Asterisks next to lines indicate a significant change from one phase of the breeding period to the next within a group of females. Samples included 15 primiparous yearlings, 11 primiparous adults, and 13 multiparous adults during gestation, 15 primiparous yearlings, 16 primiparous adults, and 31 multiparous adults during lactation, and 12 primiparous yearlings, 16 primiparous adults, and 21 multiparous adults during the post-emergence period.

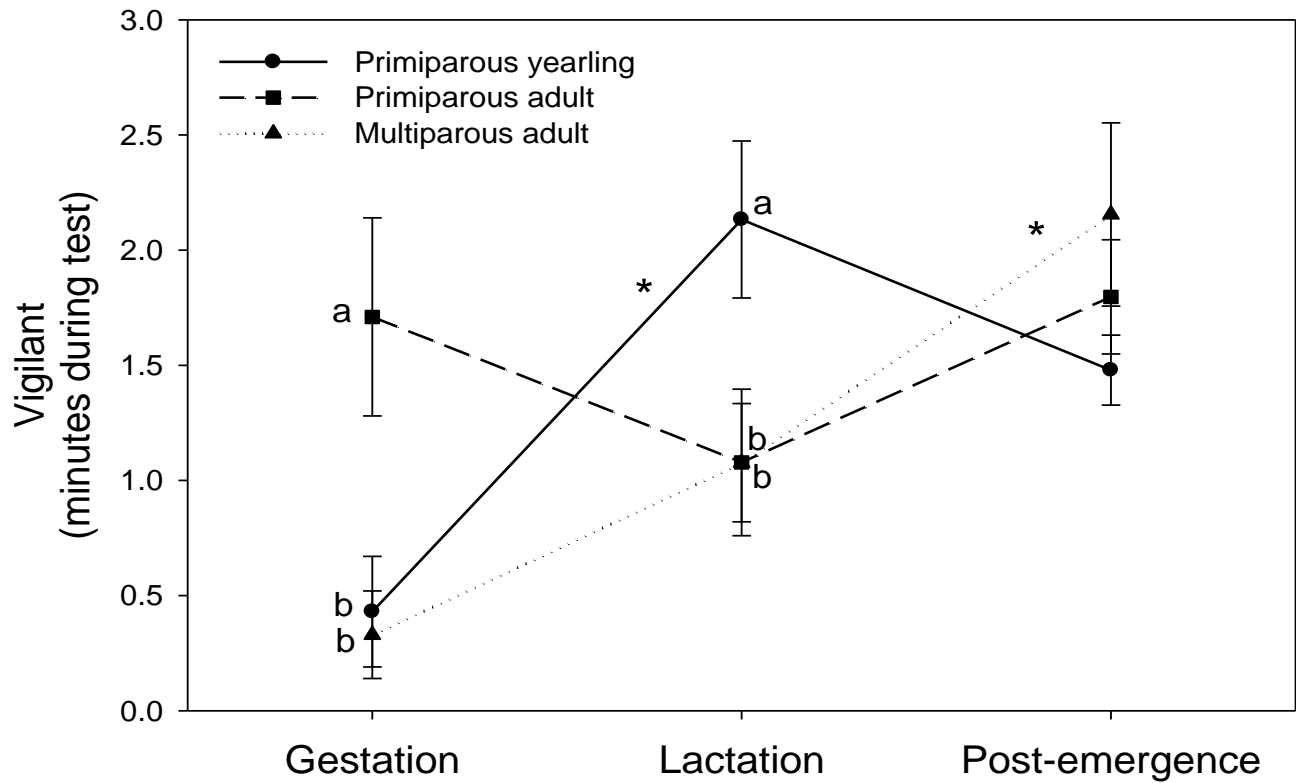


Figure 4. Time spent by female *S. beldingi* behaving vigilantly toward a caged male intruder during a 10-minute behavioral test. Different lower-case letters next to symbols indicate a significant difference between groups of females within a phase of the reproductive period. Asterisks next to lines indicate a significant change from one phase of the breeding period to the next within a group of females. Samples included 15 primiparous yearlings, 11 primiparous adults, and 13 multiparous adults during gestation, 15 primiparous yearlings, 16 primiparous adults, and 31 multiparous adults during lactation, and 12 primiparous yearlings, 16 primiparous adults, and 21 multiparous adults during the post-emergence period.

greater among primiparous adults than multiparous adults or primiparous yearlings during gestation but was significantly greater among primiparous yearlings than among primiparous adults or multiparous adults during lactation (Fig. 4). Vigilant behavior increased significantly from gestation to lactation among primiparous yearlings, and from lactation to the post-emergence period among multiparous adults.

The amount of time females watched caged males during intruder tests did not vary over the course of the breeding period (Fig. 5, ANOVA, $F_{2,141} = 0.182$, $P = 0.834$), or among parity groups (Fig. 5, ANOVA, $F_{2,141} = 0.922$, $P = 0.400$). However, there was a significant interaction between stage of the breeding period and parity class (Fig. 5, ANOVA, $F_{4,141} = 2.913$, $P = 0.024$), indicating a difference in the pattern of change in watching behavior among groups of females. In particular, the watching behavior of primiparous yearlings during gestation was significantly greater than that of primiparous or multiparous adults and decreased significantly from gestation to lactation (Fig. 5).

The amount of time females were indifferent toward males during intruder tests varied significantly over the breeding period (Fig. 6, ANOVA, $F_{2,141} = 4.249$, $P = 0.016$), and among parity groups (Fig. 6, ANOVA, $F_{2,141} = 5.055$, $P = 0.008$). There was also a significant interaction between phase of the breeding season and parity (Fig. 6, ANOVA, $F_{4,141} = 7.186$, $P < 0.001$), indicating that patterns of change in indifferent behavior varied among groups of females during the breeding period. The indifferent behavior of multiparous females was significantly greater than that of primiparous adults and yearlings during gestation, and greater than that of primiparous yearlings during lactation; however, by the post-emergence period, there was no

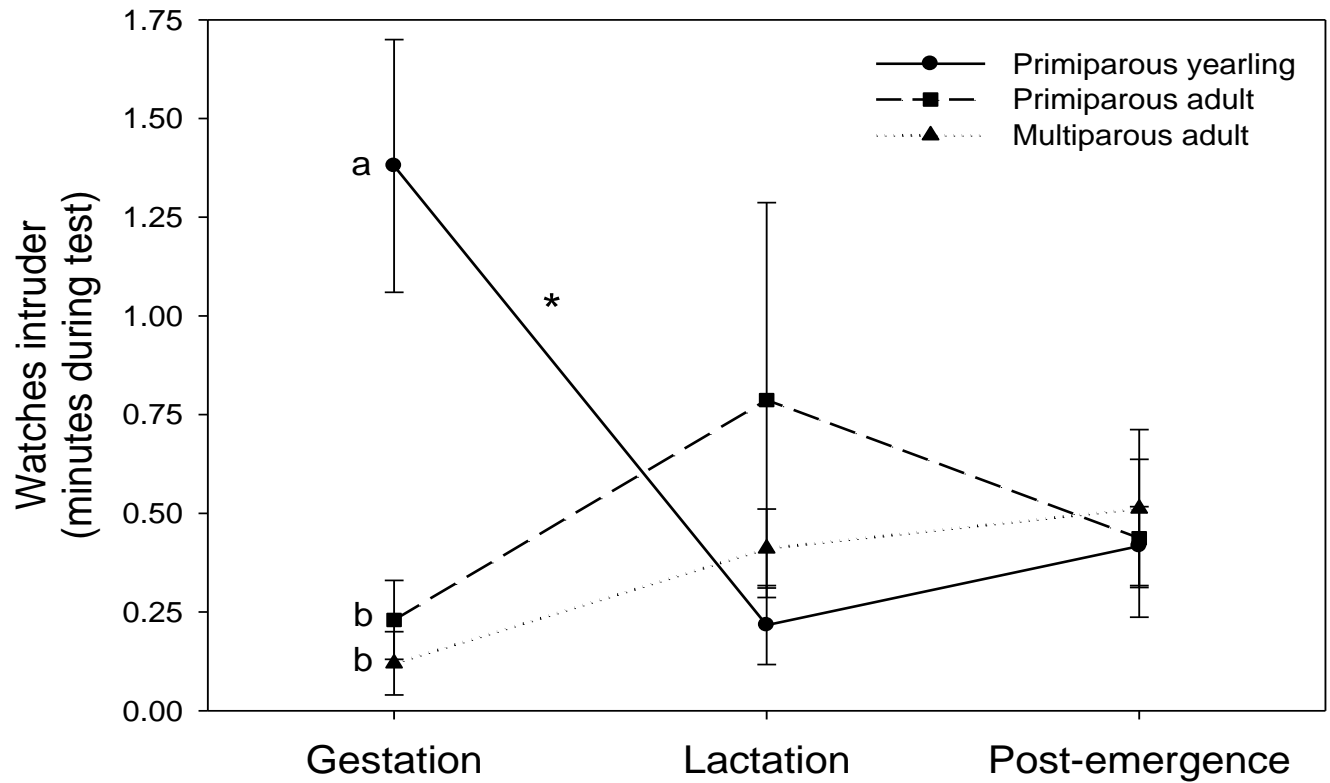


Figure 5. Time spent by female *S. beldingi* behaving aggressively toward a caged male intruder during a 10-minute behavioral test. Different lower-case letters next to symbols indicate a significant difference between groups of females within a phase of the reproductive period. The asterisks next to a line indicates a significant change among primiparous yearlings from gestation to lactation. Samples included 15 primiparous yearlings, 11 primiparous adults, and 13 multiparous adults during gestation, 15 primiparous yearlings, 16 primiparous adults, and 31 multiparous adults during lactation, and 12 primiparous yearlings, 16 primiparous adults, and 21 multiparous adults during the post-emergence period.

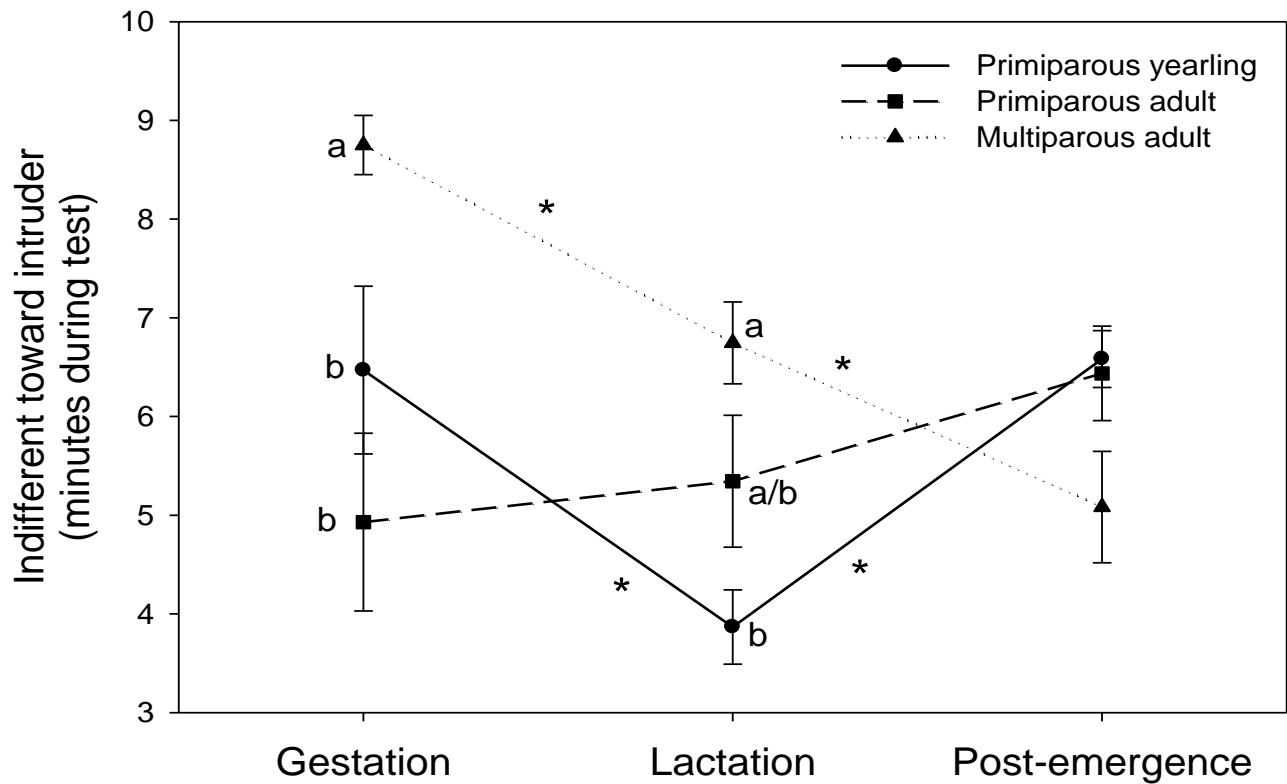


Figure 6. Time spent by female *S. beldingi* behaving indifferently toward a caged male intruder during a 10-minute behavioral test. Different lower-case letters next to symbols indicate a significant difference between parity groups within a phase of the reproductive period. Asterisks next to lines indicate a significant change from one phase of the breeding period to the next within a group of females. Samples included 15 primiparous yearlings, 11 primiparous adults, and 13 multiparous adults during gestation, 15 primiparous yearlings, 16 primiparous adults, and 31 multiparous adults during lactation, and 12 primiparous yearlings, 16 primiparous adults, and 21 multiparous adults during the post-emergence period.

significant variation among parity groups in indifferent behavior (Fig. 6). Among multiparous females, indifferent behavior declined from gestation to lactation and again from lactation to the post-emergence period, whereas among primiparous yearlings, indifferent behavior declined from gestation to lactation, but then increased from lactation to the post-emergence period (Fig. 6).

DISCUSSION

Early in the breeding season multiparous females spent more time behaving indifferently to caged intruders than primiparous females, suggesting that they were better able to assess the testing situation and determine that a caged intruder was not a threat. Early in the breeding season, primiparous adults spent more time near the intruder, more time investigating the intruder, and more time acting aggressively and vigilantly toward the intruder than did primiparous yearlings and multiparous adults. However, as the breeding season progressed, these active responses of primiparous adults to the intruders decreased. Early in the breeding season, primiparous yearling spent more time passively watching the intruder than did primiparous and multiparous adults. In the middle of the breeding season, primiparous females had more active responses toward the intruder, spending more time investigating the intruder and behaving vigilantly than primiparous or multiparous adults. By the end of the breeding season, responses of primiparous yearlings did not differ significantly from those of primiparous or multiparous adults.

Overall, results of this study suggest that both life and maternal experience influence how reproductive females respond to a potentially threatening situation. Female squirrels with both life and maternal experience appear able to quickly assess the threat level of a situation and respond appropriately. Females with life experience but no maternal experience appear to respond intensively to a potential threat. Although this might waste energy if the threat were not real, it might enhance survival of offspring if the threat were an indication of actual danger. Females, with little life experience and no maternal experience appear to respond passively to a possible threat (e.g., by watching), and as they gain life experience respond more actively. The

similarity in responses among different groups of females by the end of the breeding period supports the idea that experience raising young influences the ability to assess and respond appropriately to a situation.

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